NANOFLAGELLATES OF EAST PACIFIC COASTAL WATERS: MORPHOLOGY, TAXONOMY, AND BIOGEOGRAPHY OF WEAKLY CALCIFIED COCCOLITHOPHORIDS (PRYMNESIOPHYCEAE)

Helge Abildhauge THOMSEN[1] and Kurt R. BUCK[2]

[1] Department of Phycology, Botanical Institute, University of Copenhagen
Øster Farimagsgade 2D, DK-1353 Copenhagen K, Denmark
Fax: +45 35 32 23 21. E-mail: HelgeAT@Bot.KU.DK
[2] Monterey Bay Aquarium Research Institute, Box 628, Moss Landing,
CA 95039, USA. E-mail: BUKU@MBARI.ORG

Correspondence should be addressed to the first author

ABSTRACT — A transmission electronmicroscopical examination of whole mounts of cells prepared from samples collected off central California, September 1989, from the Sea of Cortez, (Mexico), January 1990, and from San Juan Islands, Washington, August 1983, has led to the identification of 7 taxa of weakly calcified nanoflagellates, the majority of which are related to forms which have their main distribution in polar regions.

RÉSUMÉ — Un examen, en microscopie électronique à transmission, de cellules entières préparées à partir d'échantillons prélevés au large de la Californie centrale, en septembre 1989, en mer de Cortez (Mexique), en janvier 1990, et aux îles San Juan, Washington, en août 1983, a conduit à l'identification de nanoflagellés faiblement calcifiés dont la majorité est apparentée à des formes distribuées principalement dans les régions polaires.

KEY WORDS: biogeography, central California waters, Coccolithophorids, micro-algae, morphology, nanoflagellates, Prymnesiophyceae, San Juan Islands, Sea of Cortez.

INTRODUCTION

Coccolithophorids are nanoflagellates (< 20 µm) that have calcium carbonate coccoliths located externally as a cellular periplast. Coccoliths may be robust and have complex crystallographic structure, or may be more weakly calcified and possess a relatively simple structure. Due to the refractive nature of robust coccoliths they are frequent objects of study and are useful as markers of oceanic water masses and climate change. Coccolithophorids have been shown to be more diverse and abundant with

increasing temperature and they have been considered to be absent at temperatures below 2° C. Whilst this has generally been found to be true, a series of papers by Manton and co-workers (Manton & Oates, 1975 *et seq.*) and Thomsen and co-workers (Thomsen, 1980a *et seq.*) have demonstrated the presence of a polar community of nanoplanktonic and weakly calcified coccolithophorids that thrive at temperatures as low as -2° C. In recent papers (Thomsen, 1986; Garrison & Thomsen, 1993; Marchant & Thomsen, 1994) it has been reported that the cold water coccolithophorid community consists exclusively of non-photosynthetic forms.

During September 1989 we had the opportunity to participate in an oceanographic cruise in the waters off central California allowing us to contribute new data on the biogeography, morphology and taxonomy of marine coccolithophorids from genera which are otherwise mostly associated with polar regions (Thomsen, 1981; Thomsen *et al.*, 1988). This is the third paper in a series of reports on the biodiversity of nanoflagellates in central Californian waters (see also Thomsen *et al.*, 1991a; Thomsen & Buck, 1998). This paper additionally includes material from other localities on the fringes of the Pacific Ocean, viz. San Juan Island, Washington, and the Sea of Cortez, Mexico.

The present paper reports on the finding of specimens identical to or related to members of the polar community at temperate and sub-tropical Pacific Oceanic sites. Recently documented life-histories of polar coccolithophorids combine e.g. species from the genus *Papposphaera* with species from the genus *Turrisphaera* (Thomsen *et al.*, 1991b). Due to this uncertainty we will refrain from formally describing new taxa, with the exception of *P. bourrellii* sp. nov.

MATERIALS AND METHODS

Water samples were collected from positions off California (Fig. 1) during the R/V "Point Sur" primary productivity cruise number 8 (PP8), September 25-30, 1989, and here the origin (station number) of material selected for publication is indicated in the legends. A 5 liters Niskin bottle was used to obtain the surface samples. Deeper samples were additionally collected from some stations. The nanoflagellates were, in most cases, concentrated by means of centrifugation of approx. 200 ml of prefiltered (mesh size 20 µm) seawater from each station. Light and electron microscopical whole mounts were made according to established procedures (Moestrup & Thomsen, 1980; Thomsen, 1982). Whole mounts for TEM were shadowcast with chromium. The microscope used was a JEOL 100B at the electron microscopical facility at University of California at Santa Cruz.

Surface water samples from the San Juan Islands, Washington, were collected in August 1983, at localities in the vicinity of Friday Harbor, Turn Island, Knob Island and McConnell Island (approx. 123° W, 48.5° N). The protocol was similar to that described above, with the exception that the prefiltering screen used had a mesh size of 40 µm. The whole mounts were shadowcast with gold-palladium and examined on a JEOL T7 electron microscope at the Botanical Institute, University of Copenhagen.

Samples from the Sea of Cortez, were collected at Bahia de Los Angeles, Mexico (*ca* 29° N, 113.5° W) during January 1990. The water samples collected were processed in the trunk of a rental car, using ■ sequential filtration on 3 µm Millipore filters followed by centrifugation of prefiltered (20 µm) surface water. The whole mounts were shadowcast with chromium and examined on a JEOL 100S electron microscope at the Botanical Institute, University of Copenhagen.

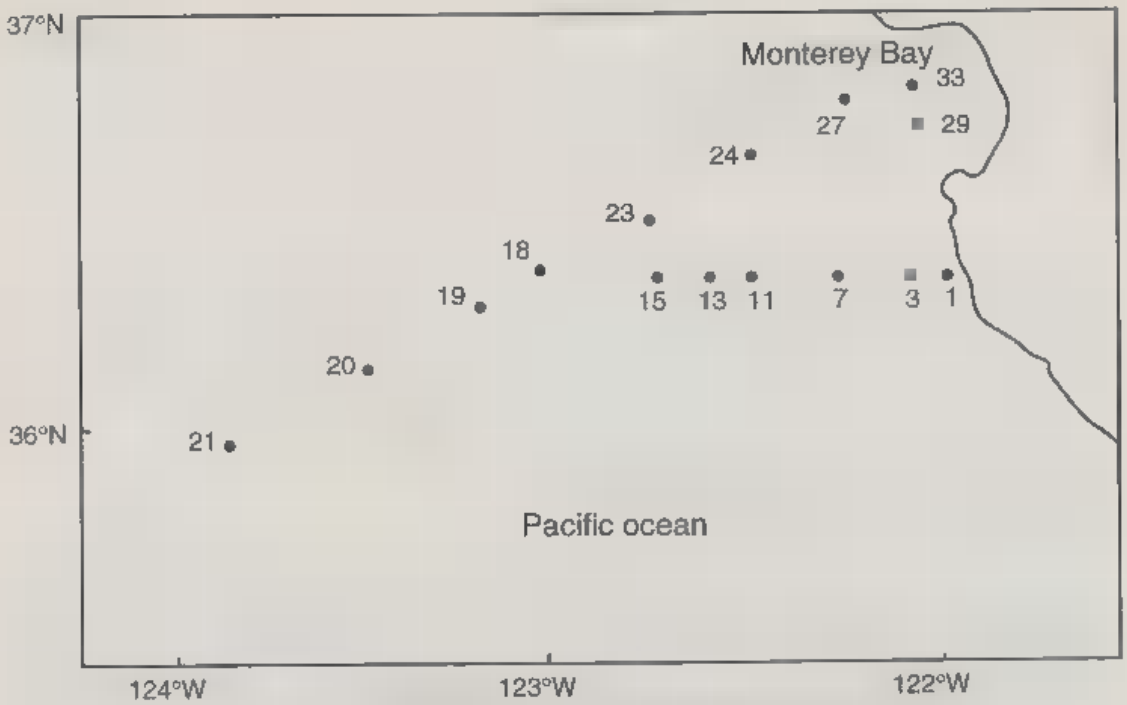


Fig. 1. Station map from central California waters.

OBSERVATIONS

In describing the taxa we follow taxonomic recommendations as suggested by Jordan *et al.* (1993), and the classification of extant Haptophyta as proposed by Jordan & Green (1994). The terminology used is in accordance with Jordan *et al.* (1995).

Class Prymnesiophyceae
Order Prymnesiales
Family Pappospharaceae

***Papposphaera* Tangen**

This genus possesses pappoliths of one type only. A "pappolith" (Norris, 1983) is a heterococcolith with a calcified rim in the form of a narrow, slightly flaring wall composed of elements of two alternating types (Jordan *et al.*, 1995). The base plate calcification is weakly developed and variable among the species presently allocated to the genus. A central process is often present. Thomsen *et al.* (1991b) showed that species of *Papposphaera* and species of *Turrisphaera* are life history stages of a single organism. This conclusion was based on the observation of single, naturally occurring cells possessing both the pappoliths, and turriform holococcoliths characteristic of *Turrisphaera*. The genus *Papposphaera* at present comprises the type species *P. lepida* Tangen ("*Turris-*

phaera" phase unknown), *P. arctica* (Manton *et al.*) Thomsen *et al.* (syn. *Papposphaera sarion* Thomsen); *P. borealis* (Manton *et al.*) Thomsen *et al.* (syn. *Papposphaera sagittifera* Manton *et al.*), *P. obpyramidalis* Thomsen ("*Turrisphaera*" phase unknown), *P. polybotrys* (Thomsen) Thomsen *et al.* ("*Papposphaera*" phase unknown), *P. simplicissima* Thomsen ("*Turrisphaera*" phase unknown), *P. thomsenii* Norris ("*Turrisphaera*" phase unknown) (Manton *et al.*, 1976a,b; Norris, 1983; Tangen, 1972; Thomsen, 1980b; Thomsen *et al.*, 1988, 1991b).

Species of "*Turrisphaera*" have proved very difficult to identify from TEM whole mounts, and it has been obvious that a reliable discrimination of taxa is often not possible, unless supported by the simultaneous discovery (e.g. as combination cells) of the *Papposphaera* counterpart in the *Turrisphaera-Papposphaera* life history. The present material did not comprise any combination cells, which is one reason why we have tentatively to group the "*Turrisphaera*"-cells encountered under provisional headings.

***Papposphaera lepida* Tangen, Figs 2-8.**
(description of "*Papposphaera*" phase)

The original description of this species (Tangen, 1972) was based on light and scanning electron microscopy. The cell body diameter was 4.5-7 µm, whereas the diameter of the coccosphere ranged from 11-16 µm. Two flagella, 2-3 times the diameter of the coccosphere, but no haptonema, were seen in the type material. Tangen (1972) cautiously stated that the genus is "apparently" characterised by having 2 chloroplasts. The coccoliths (50-100 per cell) are all of the same basic type with an elliptical to subcircular base plate (long axis: 0.8-1.4 µm) and a central process (2-3.9 µm long) which terminates in a wide, subcircular to four-lobate, funnel-shaped structure (diameter 1.1-2.1 µm). Four decurrent ridges from the central process form a distinct, calcified cross on the base plate. Limited by the resolution of the SEM Tangen (1972) was only able to describe the scale rim (0.2-0.35 µm high) as consisting of 18-24 thin plate elements (pentagonal) and with an irregularly toothed upper rim.

Transmission electron micrographs of South African material published by Manton & Oates (1975, figs 3-4) added significant details to the first description of *P. lepida*. A second ring of narrow, rod-shaped crystallites was found to be part of the base plate rim. An additional feature clearly resolved in the transmission electron microscope was the radiating ridges of the organic, subtending scale. More recently *P. lepida* (as *Papposphaera* sp.) from the North Pacific Central Gyre was also described and illustrated (TEM) (Hoepffner & Haas, 1990). These authors hesitated to identify the material examined as *P. lepida* because the protoplast comprised coccoliths of different sizes, though apparently with the same overall morphology, and also because of the finding of special structural features interlocking the process shaft and the distal plates. Other species of *Papposphaera*, viz. *P. borealis* (syn. *P. sagittifera*), have been found to be characterised by a pronounced polarity in the development of the single type of coccolith present. This variability appears to be mostly in size, with more fully developed and larger coccoliths typically clustering at the apical pole of the cell. We also believe that *P. lepida* sometimes shows a similar gradation in coccolith size from one end of the cell to the other, and that this is in fact what Hoepffner & Haas (1990) observed. The interlocking, structural details, e.g. a small wristlet-like expansion of the distal part of the process shaft, and a displacement (eccentricity) of the individual quadrants to produce a small central and angular hole that matches the "wristlet", was also illustrated but not commented on by Manton &

Oates (1975). It was obviously beyond the resolution power of the SEM used by Tangen (1972) when examining the type-material. In our opinion the North Pacific Gyre material examined by Hoepffner & Haas (1990) can be unequivocally identified as *P. lepida*.

The Californian specimen (Figs 2-4) is similar to previously examined material (Tangen, 1972; Manton & Oates, 1975; Hoepffner & Haas, 1990), and shares with the cells from the North Pacific Gyre a pronounced polarity in development of the individual coccoliths. There is a gradual decrease in distal quadrant edge length from the apical pole (2.2 µm) towards the antapical pole (1.3 µm). Details of the interlocking devices, *i.e.* a wristlet-like, cross-shaped termination of the central process, and slightly offset quadrants producing a matching cavity (Fig. 4) are virtually indistinguishable from those illustrated by Hoepffner & Haas (1990). The distal appendage is subquadrangular on apical pole coccoliths (Fig. 3, arrow) and with rounded corners, while approximately circular on coccoliths at the antapical cell end (Fig. 3, arrowhead).

Cells from Mexico (Figs 5-8) initially appear sufficiently different from material cited above to warrant description as an independent species. However, despite the conspicuous difference in shape of the distal appendage (perfect squares in Mexican cells) the presence within the very same periplast of a single coccolith (Fig. 8, arrowhead) with a quasi-discoid appendage, indistinguishable from e.g. those on Californian cells (Fig. 3), suggests that a species distinction cannot be based on this particular feature. It should be emphasised that the pappoliths on the Mexican cells in all other aspects (viz. scale rim and base plate calcification (Fig. 7, arrow), interlocking (Fig. 6) of shaft and distal appendage) are virtually indistinguishable from *P. lepida* pappoliths studied previously. Also, the Mexican cells (Figs 5, 8) display a pronounced reduction in size (see Fig. 3) from the apical pole (edge of distal quadrant: 1.5 µm; length of shaft: 2 µm) towards the antapical pole (edge of distal quadrant: 1 µm; length of shaft: 1 µm). A haptonema (Fig. 5, arrow) has not previously been observed in specimens identified as *P. lepida*.

BIOGEOGRAPHY: *Papposphaera lepida* is infrequently recorded. However, it is widely distributed with a concomitantly wide temperature range, indicating that this taxon is eurythermic. It appears to thrive in sea water of oceanic salinity and is thus best characterised as being stenopolyhaline. Abundance estimates based on cell enumerations of preserved material examined with the inverted microscope (Tangen, 1972) or SEM (Samtleben & Schröder, 1992) were 10^3 and 3×10^2 cells l^{-1} respectively. Previous findings: Nordåsvatnet (type locality), Western Norway (Tangen, 1972; salinity mostly > 27 PSU; temperature 6.5-20.5° C); Caribbean Sea (Throndsen, 1972; as Coccolithophorid sp. 2; see Tangen, 1972); North-West Africa (Heimdal, pers. com. in Tangen, 1972); Cape Town (Manton & Oates, 1975; 9-10° C); North Atlantic (Okada & McIntyre, 1977; St. "Charlie"); Iceland Plateau (St. 9) in the proximity of Jan Mayen (Samtleben & Schröder, 1992; approx. 2-6° C); Denmark (Thomsen, unpublished results; January 1976; 2.7° C, 26.3 PSU; June 1976; 6.3° C, 31.4 PSU (15 metres depth); Phuket, Thailand (Thomsen, unpublished results; August 1981; 27° C, 35 PSU).

***Papposphaera bourrellii* sp. nov., Figs 9-16.**
(description of "*Papposphaera*" phase)

DIAGNOSIS: The cell is spherical, *ca* 4 µm in diameter, with 2 almost equally long flagella (*ca* 20 µm) and a significantly shorter, coiling haptonema (Fig. 13, arrow). Diam. of coccosphere *ca* 13 µm. Coccoliths of one type (pappoliths). The elliptical base plate

(0.8 × 0.5 µm) is calcified along the rim and in a cruciform pattern on the scale surface (Fig. 14). The rim calcification consists of 2 series of crystallites (Fig. 14), *i.e.* small rod-shaped crystallites (0.15 × 0.03 µm), and larger polygonal elements (ca 0.15 µm) that form the upright, irregularly toothed rim. The shaft of the central process measures ca 3.5 µm in length and supports a distal, calyx-like appendage, consisting of 4 rhomboidal, sepal-like structures (Fig. 15). Each of these measure ca 0.6 × 0.3 µm. The interconnection between the shaft and the "calyx" is facilitated by a wristlet-like distal thickening of the shaft (Fig. 15, arrow). The "*Turrisphaera*"-phase of this taxon is presently unknown.

Holotype specimen (iconotype) and type locality: Fig. 13 from San Juan Islands (McConnell Island; < 40 metres depth), Washington, USA, 8 August 1983.

Cellula sphaerica, circa 4 µm diametro, flagellis binis, subaequalibus et haptonemate manifeste breviori instructa. Diametrum coccosphaerae circa 13 µm. Coccilithi monomorphi (pappolithi). Lamina basalis elliptica secus marginem et in superficie squamae cruciforme calcificata. Calcificatio marginis e seriebus duabus crystallitarum, i.e. crystallitis parvis bacilliformibus et elementis amplioribus polygonalibus, marginem erectam irregulariter dentatam formantibus composita. Scapus processus centralis circa 3.5 µm longus, appendicem calyciformem distalem, e 4 compaginibus rhomboideis, sepaloideis compositam sustineus. Compagines circa 0.6 × 0.3 µm metientes. Junctura scapi et calycis crassitie annulari scapi distingitur. Phasis Turrisphaera hujus speciei ignota.

This species is named in honour of our late colleague, Prof. Dr Pierre Bourrelly, Muséum National d'Histoire Naturelle, Paris, who has for decades remained a key figure in research related to Chrysophyta *sensu lato*.

Pappasphaera bourrellii sp. nov. is easily distinguished from all previously described species of *Pappasphaera*, by the appearance of the calyx-like distal appendage. Another morphological characteristic which may be unique to this taxon relates to the calcification of the pappolith base-plates. The cross-shaped calcification, also known from the type-species *P. lepida*, and e.g. *P. arctica* (syn. *P. sarion*) and *P. obpyramidalis*, is also clearly present in *P. bourrellii* (Fig. 14). However, it additionally appears from Fig. 14 that in this species a major part of the base-plate surface area is covered by calcified, thin, rectangular plates. This fact has to be verified through the study of additional and better oriented coccoliths than those presently available.

In addition to the type locality, we have also found *P. bourrellii* in samples from California (Figs 9-12) and Mexico (Fig. 16; identification based on LM observations only). The Californian material is indistinguishable from the McConnell Island type material with the possible exception of the apparent lack of the extended base-plate calcification (compare Fig. 10 and Fig. 14).

This taxon has been previously illustrated (as *Pappasphaera* sp.) in Thomsen *et al.* (1994, figs 2-3).

***Pappasphaera* sp. 1, Fig. 17.**
Description of "*Pappasphaera* phase"

A fragment of a protoplast (Fig. 17) with coccoliths (pappoliths) unmistakably reminiscent of those of *Pappasphaera* spp. was encountered in a Mexican sample. The central process is elaborate and clearly consists of 4 parallel lines of crystallites that

continue in a cruciform pattern across the base-plate towards the rim of the coccolith. Large angular crystallites from the scale rim calcification are visible in Fig. 17 (arrow). The distal appendage consists of 4 quasi-rectangular, diverging plates. This material obviously represents a new species of *Papposphaera*. The formal taxonomic description is postponed until complete cells become available.

***Papposphaera* sp. 2, Figs 18-22.**
Description of "*Turrisphaera* phase"

This species possesses slender and cylindrical, turriform coccoliths which are each constructed from numerous, reticulately arranged hexagonal plates with a diameter of approximately 0.1 µm (Fig. 19). The cell (Fig. 22) has 2 almost equally long flagella (approx. 22.5 and 25 µm) and a somewhat shorter haptonema (approx. 15 µm). In some cells (e.g. Fig. 18 from Mexico and probably Fig. 22 from California) the coccoliths are relatively symmetrical structures, slightly swollen towards the distal tip, and often with some difference in overall size (1-2 µm) from one end of the cell to the other. In other cells from the same two localities (Figs 20-21) the coccoliths loose their overall symmetry and become finger-like structures. Non-mineralised, organic underlayer scales are visible in Fig. 21 (arrow). In this particular cell, part of the calcified periplast has broken away, thus exposing the underlayer of scales. At high magnification they are oval (0.5 × 0.4 µm), and with a distinct rim and a little pronounced surface pattern of ridges.

This taxon was previously illustrated (as *Turrisphaera* sp.) in Thomsen *et al.* (1994, fig. 5).

***Papposphaera* sp. 3. Figs 23-26**
Description of "*Turrisphaera* phase"

We identified a second, distinct species of "*Turrisphaera*" in the Californian material (Figs 23-26). In these organisms there is a significant difference between apical pole coccoliths and those found elsewhere in the periplast. The latter are fairly short (1.5 µm long) and symmetrical, turriform coccoliths with a conspicuous median constriction (Fig. 24, arrow). The apical pole coccoliths (3.0 µm long) are characterised by a conspicuous, unilateral proliferation of the distal end of each coccolith. Details of the proximal parts of apical pole coccoliths cannot at present be accounted for. The flagella (Fig. 26) are almost equally long (*ca* 25 µm) and the haptonema (Figs 24, 26) significantly shorter (*ca* 15 µm). Typical cell dimensions are 5-6 µm.

This species most closely resembles the "*Turrisphaera*" phase of *P. polybotrys* (Thomsen, 1980b), which is similarly characterised by two types of coccoliths of which those clustering at the apical pole have conspicuous unilateral proliferations. The main differences relate to the overall shape of the coccoliths. The apical pole coccoliths of *P. polybotrys* are elongate and narrower than those seen in the Californian material. The goblet-shaped coccoliths of *P. polybotrys* also differ in small details, e.g. with regard to the median constriction of each coccolith. It is possible that the West Greenland type material of *P. polybotrys* and the Californian material do represent the very same taxon, but, the final proof of conspecificity must come from a comparison of the *Papposphaera* phases of these populations, and thus awaits the finding of combination cells. A combination cell involving *Turrisphaera polybotrys* and *Papposphaera* sp. has been encountered in West

Greenland waters (Thomsen *et al.*, 1991b). However, due to the preservation stage of the material and the orientation of the pappoliths it was not possible to verify the identity of the *Papposphaera* counterpart of the "*Turrisphaera*" phase of *P. polybotrys*. Hoepffner & Haas (1990) found a *Turrisphaera* sp. in samples from the North Pacific central gyre which shares with the Californian material examined here the possession of apical pole coccoliths with distinctive, unilateral, distal proliferations of the turriform coccolith. The single cell from the central gyre (Hoepffner & Haas, 1990) was compared with two forms of "*Turrisphaera*" (viz. *P. borealis* and *P. arctica*) described by Manton *et al.* (1976b), but is in fact more closely reminiscent of *P. polybotrys* than the cells illustrated from Californian waters and discussed above.

Pappomonas Manton & Oates

The *Pappomonas* coccosphere is composed of more than one type of coccolith, each distributed in a specific area of the coccosphere. All coccoliths are pappoliths. The main differences between coccoliths in different parts of the periplast relate to: 1) the shape of the base-plate (circular in apical pole coccoliths) and oval in all other coccoliths, 2) the base-plate calcification (cruciform in apical pole coccoliths and in others consisting of rectangular bars of crystallites arranged roughly parallel to the long axis of the plate), and 3) the presence or absence of a central process. Coccoliths at the apical pole always carry a very conspicuous central processes. Coccoliths at the antapical pole sometimes possess somewhat shorter and also morphologically simpler central processes. The genus at present comprises 4 taxa, viz. *P. flabellifera* Manton & Oates, 1975 var. *flabellifera*; *P. flabellifera* var. *borealis* Manton et al., 1976; *P. virgulosa* Manton & Sutherland, 1975; and *P. weddellensis* Thomsen *in* Thomsen *et al.*, 1988. It was reported by Thomsen *et al.* (1991b), that species of *Pappomonas* and species of *Trigonaspis* Thomsen (Thomsen, 1980a) sometimes form combination cells, which indicates that the taxa involved (*P. flabellifera* var. *borealis* and *Trigonaspis* cf. *diskoensis* Thomsen, 1980) are different phases of the same life-cycle. The genus *Trigonaspis* Thomsen, 1980, is closely related to *Turrisphaera*, the main difference being the shape of the crystallites, which are triangular in *Trigonaspis*. This genus currently comprises three species, viz. *T. diskoensis* Thomsen, 1980; *T. minutissima* Thomsen, 1980; and *T. melvillea* Thomsen *in* Thomsen *et al.*, 1988. The genus *Pappomonas* takes priority over *Trigonaspis*. However, the formal new combination of taxa should be postponed until more examples of *Pappomonas/Trigonaspis* combination cells, in which both phases can be unequivocally identified, have been documented. Preliminary results (Østergaard, 1993) indicate that *P. virgulosa* form combination cells with *Balaniger balticus* Thomsen & Oates (Thomsen & Oates, 1978).

Pappomonas flabellifera Manton & Oates var. *flabellifera*. Figs 27-29

This taxon was found both in Californian (Figs 28-29) and Mexican water samples (Fig. 27). In all cases the cells were found to be in complete morphological and dimensional agreement with previously examined material. Antapical cell end pappoliths sometimes possessed reduced and morphologically simplified central processes (Figs 27-28). The micrograph (Fig. 29) has been previously published by Thomsen *et al.* (1994, fig. 9).

BIOGEOGRAPHY: *P. flabellifera* var. *flabellifera* is currently known from South Africa (Manton & Oates, 1975), West Greenland (Thomsen, 1981), Denmark (Thomsen, unpublished results). California and Mexico.

Genera *incertae sedis*

***Polycrater* Manton ■ Oates, 1980.**

This genus is unusual not only because of a unique morphology of the individual coccolith, but also in having a microcrystalline substructure based on calcium carbonate as aragonite instead of the more usual calcite (Manton & Oates, 1980). A coccolith consists of 4 petal-like segments (Fig. 31; arrows) forming a bowl, which on the convex side supports a heavily calcified and cruciform, lobed structure (Fig. 31; arrowheads) in which each arm is lined up exactly underneath the bordering lines between the petal-like segments. Contrary to other genera considered, which have their main distribution within temperate and polar regions, the genus *Polycrater* is apparently restricted to sub-tropical and tropical regions.

***Polycrater galapagensis* Manton & Oates, 1980, Figs 30-31.**

The Californian material exactly matches the type specimen from the Galapagos Islands (Manton & Oates, 1980). The cells illustrated here also document the presence of a haptonema (Fig. 30; arrow). When completely stretched out the haptonema is approximately half the length of the flagella. The micrograph (Fig. 30) has been previously published in Thomsen *et al.* (1994, fig. 6).

BIOGEOGRAPHY:*P. galapagensis* is recorded from the Galapagos Islands (Manton & Oates, 1980; 22° C and oceanic salinity), the Atlantic Ocean (Chrétiennot-Dinet, 1990), the Mediterranean Sea, Egypt (Thomsen, unpublished results), California and Mexico. This taxon appears to have a world-wide distribution limited to sub-tropical and tropical regions.

DISCUSSION

Due to past sampling being biased towards coastal and polar regions, the lightly calcified genera (e.g. *Papposphaera*, *Pappomonas*, *Turrisphaera*, *Trigonaspis*, *Wigwamma*) that need to be studied as TEM whole mounts for identification purposes, are thought to be found mainly in regions of the world oceans that border the main biogeographical provinces of coccolithophorid distribution. The present paper documents that these particular genera are also present in regions where typical coccolithophorids are abundant.

The polar representatives of the lightly calcified coccolithophorid genera have recently been shown to be aplastidic (Garrison & Thomsen, 1993; Marchant & Thomsen, 1994). This was deduced through a combination of epifluorescence and phase contrast

microscopy of recently collected specimens viewed as whole mounts for light microscopy prepared according to the procedure described by Thomsen (1982). Whether the same genera are also aplastidic when found outside regions characterised by prolonged periods of darkness needs, as a minimum requirement, to be verified from microscopy of freshly collected material in which the chlorophyll autofluorescence has not yet faded. Examination of thin sections with TEM for the presence (absence) of chloroplasts and/or food vacuoles would unequivocally both validate these earlier findings and also give a first hint on whether the physiology of these organisms is apt to change with latitude.

Considering the existence of complex life histories that link morphologically dissimilar taxa (viz. *Papposphaera-Turrisphaera*, *Pappomonas-Trigonaspis*; see Thomsen *et al.*, 1991b), it is interesting to note that the majority of the cells collected from Californian and Mexican waters can be segregated into 2 species of *Papposphaera* (*P. lepida* and *P. bourrellii*) and 2 species of "*Turrisphaera*" (*Papposphaera* sp. 2 and 3). It is, despite the present lack of combination cells, tempting to hypothesise that these 4 forms will eventually be found to represent 2 species, that for yet unknown reasons, can switch between a *Papposphaera* (heterococcolithophorid) phase and a *Turrisphaera* (holococcolithophorid) phase, a switch that is most likely linked to changes in ploidy level (Billard, 1994).

The haptophytes are important contributors to biogeochemical cycles in marine environments. They are intimately associated with sulphur and carbon fluxes, both of which are instrumental in global climate change scenarios. The importance of the organisms we describe here, weakly calcified coccolithophorids, is largely unknown. This lack of information on their ecological significance stems in large part from our inability to enumerate them. Results based on epifluorescence microscopical filter counts (EFM) do not provide information on the specific contributions by unmineralised forms (e.g. *Chrysochromulina* spp. and *Phaeocystis* spp.) and coccolithophorids, respectively. However, by using other techniques (inverted microscopy of sedimented samples) the abundance of the latter have been found to be typically within the range 10^3 – 10^4 cells l^{-1}, whereas the entire haptophyte community typically exceeds 10^6 cells l^{-1} (Thomsen *et al.*, 1994). The weakly mineralised forms treated here are generally too inconspicuous to be enumerated from settled samples. The apochlorotic nature of the cells furthermore ensures that they are likely to be incorporated as heterotrophic flagellates in the EFM count statistics, and only recognizable as members of the Prymnesiophyceae if both flagella and haptonema are well preserved. However, the examination of whole mounts of cells prepared for light microscopy has for the Antarctic indicated likely cell abundances of 1-5 × 10^3 cells l^{-1} (Marchant & Thomsen, 1994). Realizing that these organisms tend to be most abundant in polar regions, their contribution to the unicellular biomass in samples from lower latitudes is probably low. The motivation and justification for further study of these particular types of coccolithophorids is not their contribution to carbon flow, but rather the unique position of this species complex within the Prymnesiophyceae. Their lack of chloroplasts indicate that organisms reminiscent of these are potential ancestors for the entire group (Cavalier-Smith, 1994) and their participation in complex life-histories involving holo — and heterococcolithophorid stages contributes significant new knowledge on the biology of the Prymnesiophyceae.

ACKNOWLEDGEMENTS — Curt Collins of the Naval Postgraduate School, Monterey, California, generously made ship time available to us. Jane Kogelschatz assisted in the collection and processing of the samples and we thank her for her help. Funds from the Danish National Science Research Council (to HAT) and the Packard Foundation (to

Francisco Chavez, MBARI) supported participation of HAT and KRB in the R/V "Point Sur" cruise. Jonathan Krupp and Patsy Bolt (Electron Microscope Facility at the University of California Santa Cruz) provided generous assistance while samples were examined. Beatrice Booth and Rita Horner, both from the University of Washington, Seattle, very kindly organised the collecting trip to the San Juan Islands. Carol Kosman is gratefully acknowledged for her invaluable help during the examination of TEM whole mounts from Mexico. Lene Christiansen (Botanical Institute, University of Copenhagen) is acknowledged for darkroom assistance. The Latin diagnosis was kindly provided by Peter Wagner (Botanical Museum and Library, University of Copenhagen). The Systematics Association is acknowledged for permission to reproduce Figs 11, 22, 29-30 which were previously published by Thomsen *et al.* (1994).

REFERENCES

BILLARD C., 1994 — Life cycles. *In*: Green J.C. & Leadbeater B.S.C. (eds) *The Haptophyte Algae*. Systematics Association Special Volume No. 51, Clarendon Press, Oxford, pp. 167-186.

CAVALIER-SMITH, T., 1994 — Origin and relationships of Haptophyta. *In*: Green J.C. & Leadbeater B.S.C. (eds.) *The Haptophyte Algae*. Systematics Association Special Volume No. 51, Clarendon Press, Oxford, pp. 413-435.

CHRÉTIENNOT-DINET M.-J., 1990 — *Atlas du Phytoplancton Marin*. Volume III. Paris, Centre National de la Recherche Scientifique, 261 p.

GARRISON D.L. & THOMSEN H.A., 1993 — Ecology and biology of ice biota. *Berichte zur Polarforschung* 121: 68-74.

HOEPFFNER N. & HAAS L.W., 1990 — Electron microscopy of nanoplankton from the North Pacific Central Gyre. *Journal of Phycology* 26: 421-439.

JORDAN R.W. & GREEN J.C., 1994 — A check-list of the extant haptophyta of the world. *Journal of the Marine Biological Association of the United Kingdom* 74: 149-174.

JORDAN R.W., KLEIJNE A. & HEIMDAL B.R., 1993 — Proposed changes to the classification system of living coccolithophorids. III. *INA Newsletter* 15 (1): 18-22.

JORDAN R.W., KLEIJNE A., HEIMDAL B.R. & GREEN J.C., 1995 — A glossary of the extant Haptophyta of the world. *Journal of the Marine Biological Association of the United Kingdom* 75: 769-814.

MANTON I. & OATES K., 1975 — Fine-structural observations on *Papposphaera* Tangen from the southern hemisphere and on *Pappomonas* gen. nov. from South Africa and Greenland. *British Phycological Journal* 10: 93-109.

MANTON I. & OATES K., 1980 — *Polycrater galapagensis* gen. et sp. nov., a putative coccolithophorid from the Galapagos Islands with an unusual aragonite periplast. *British Phycological Journal* 15: 95-103.

MANTON I., SUTHERLAND J. & MCCULLY M., 1976a — Fine structural observations on coccolithophorids from South Alaska in the genera *Papposphaera* Tangen and *Pappomonas* Manton and Oates. *British Phycological Journal* 11: 225-238.

MANTON I., SUTHERLAND J. & OATES K., 1976b Arctic coccolithophorids: two species of *Turrisphaera* gen. nov. from West Greenland, Alaska, and the Northwest Passage. *Proceedings of the Royal Society, London, Ser. B, Biological Sciences*, 194: 179-194.

MARCHANT H.J. & THOMSEN H.A., 1994 — Haptophytes in polar waters. *In*: Green J.C. & Leadbeater B.S.C. (eds), *The Haptophyte Algae*. Systematics Association Special Volume No. 51, Oxford, Clarendon Press, pp. 209-228.

MOESTRUP Ø. & THOMSEN H.A., 1980 — Preparation of shadow-cast whole mounts. *In*: E. Gantt (ed.), *Handbook of phycological methods*. Cambridge, Cambridge University Press, pp. 385-390.

NORRIS R.E., 1983 — The family position of *Papposphaera* Tangen and *Pappomonas* Manton & Oates (Prymnesiophyceae) with records from the Indian Ocean. *Phycologia* 22: 161-169.

OKADA H. & MCINTYRE A., 1977 — Modern coccolithophores of the Pacific and North Atlantic Oceans. *Micropaleontology* 23: 1-55.

SAMTLEBEN C. & SCHRÖDER A., 1992 — Living coccolithophore communities in the Norwegian-Greenland Sea and their record in sediments. *Marine Micropaleontology* 19: 333-354.

TANGEN K., 1972 — *Papposphaera lepida*, gen. nov., n. sp., a new marine coccolithophorid from Norwegian coastal waters. *Norwegian Journal of Botany* 19: 171-178.

THOMSEN H.A., 1980a — Two species of *Trigonaspis* gen. nov. (Prymnesiophyceae) from West Greenland. *Phycologia* 19: 218-229.

THOMSEN H.A., 1980b — *Turrisphaera polybotrys* sp. nov. (Prymnesiophyceae) from West Greenland. *Journal of the Marine Biological Association of the United Kingdom* 60: 529-537.

THOMSEN H.A., 1981 — Identification by electron microscopy of nanoplanktonic coccolithophorids (Prymnesiophyceae) from West Greenland, including the description of *Papposphaera sarion* sp. nov. *British Phycological Journal* 16: 77-94.

THOMSEN H.A., 1982 — Planktonic choanoflagellates from Disko Bugt, West Greenland, with a survey of the marine nanoplankton of the area. *Meddelelser om Grønland, Bioscience* 8: 1-35.

THOMSEN H.A., 1986 — A survey of the smallest eucaryotic organisms of the marine phytoplankton. [*In*: Platt T. & Li W.K.W. (eds), *Photosynthetic Picoplankton*]. *Canadian Bulletin of Fisheries and Aquatic Sciences* 214: 121-158.

THOMSEN H.A. & BUCK K.R., 1998 — Nanoflagellates of the central California waters: Taxonomy, morphology and biogeography of primitive green nanoflagellates (Pedinophyceae, Prasinophyceae). *Deep-Sea Research II* (in press).

THOMSEN H.A., BUCK K.R. & CHAVEZ F.P., 1991a — Choanoflagellates of the central California waters: Taxonomy, morphology and species assemblages. *Ophelia* 33: 131-164.

THOMSEN H.A., BUCK K.R. & CHAVEZ, F.P., 1994 — Haptophytes as components of marine phytoplankton. *In*: Green J.C. & Leadbeater B.S.C. (eds), *The Haptophyte Algae*. Oxford, Clarendon Press, pp. 187-208.

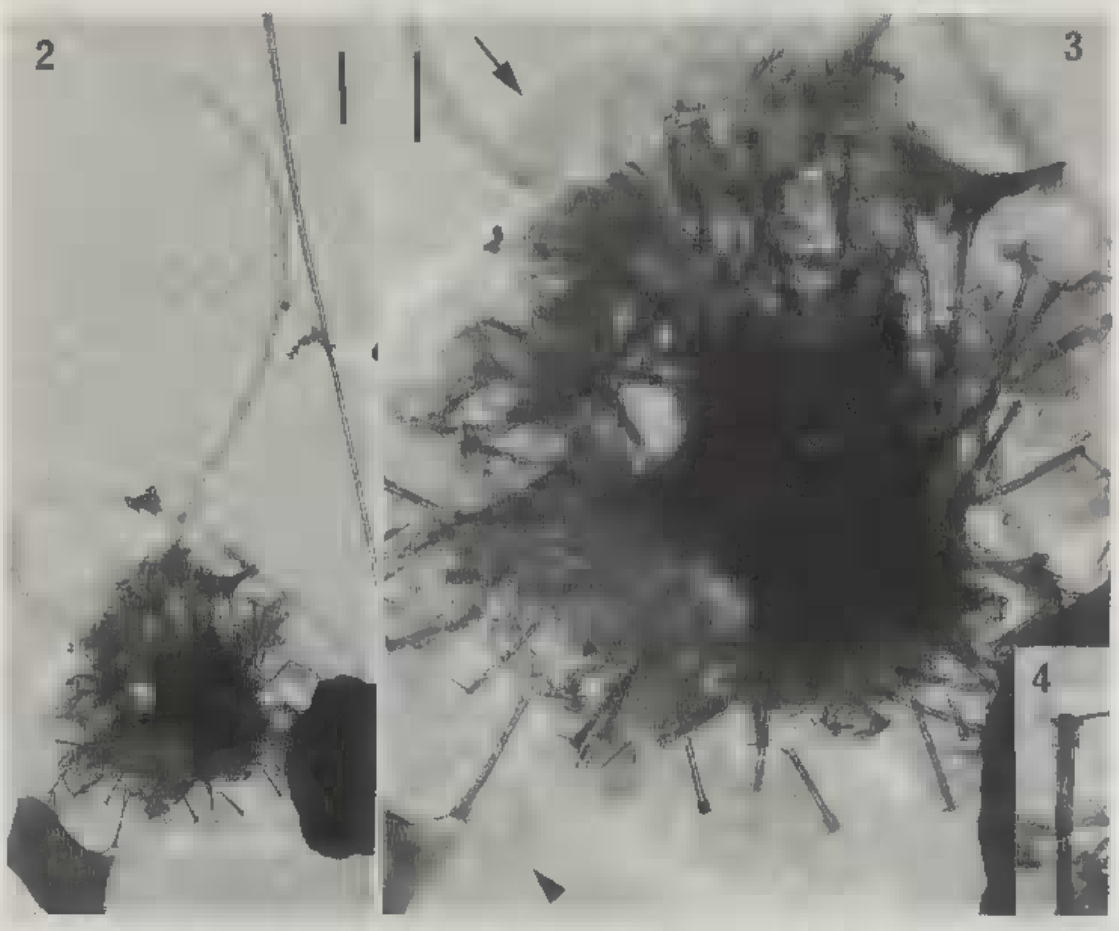
THOMSEN H.A., BUCK K.R., COALE S.L., GARRISON D.L. & GOWING M.M., 1988 Nanoplanktonic coccolithophorids (Prymnesiophyceae, Haptophyceae) from the Weddell Sea, Antarctica. *Nordic Journal of Botany* 8: 419-436.

THOMSEN H. A. & OATES K., 1978 — *Balaniger balticus* gen. et sp. nov. (Prymnesiophyceae) from Danish coastal waters. *Journal of the Marine Biological Association of the United Kingdom* 58: 773-779.

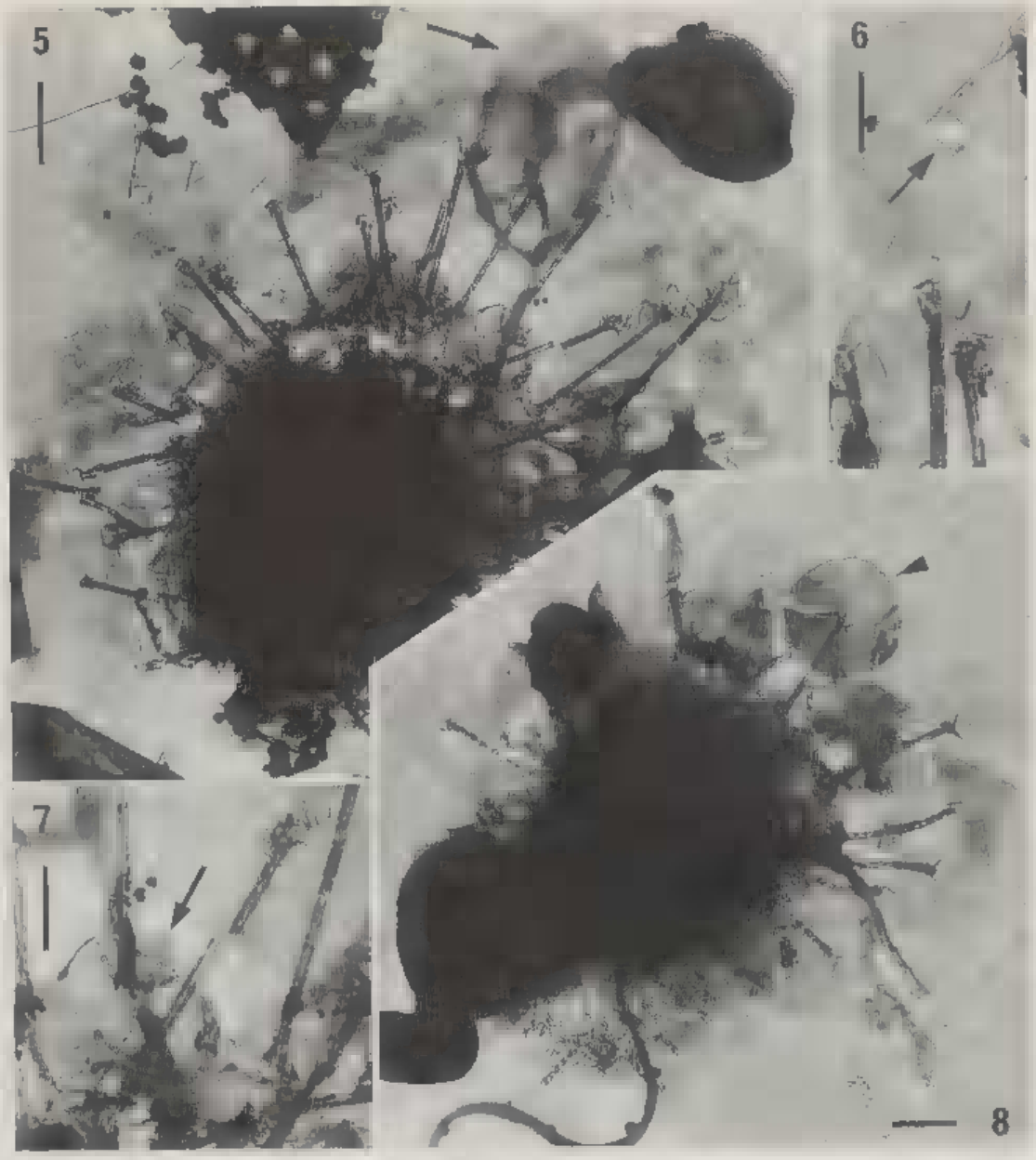
THOMSEN H.A., ØSTERGAARD J.B. & HANSEN L.E., 1991b — Heteromorphic life histories in Arctic coccolithophorids (Prymnesiophyceae). *Journal of Phycology* 27: 634-642.

THRONDSEN J., 1972 — Coccolithophorids from the Caribbean Sea. *Norwegian Journal of Botany* 19: 51-60.

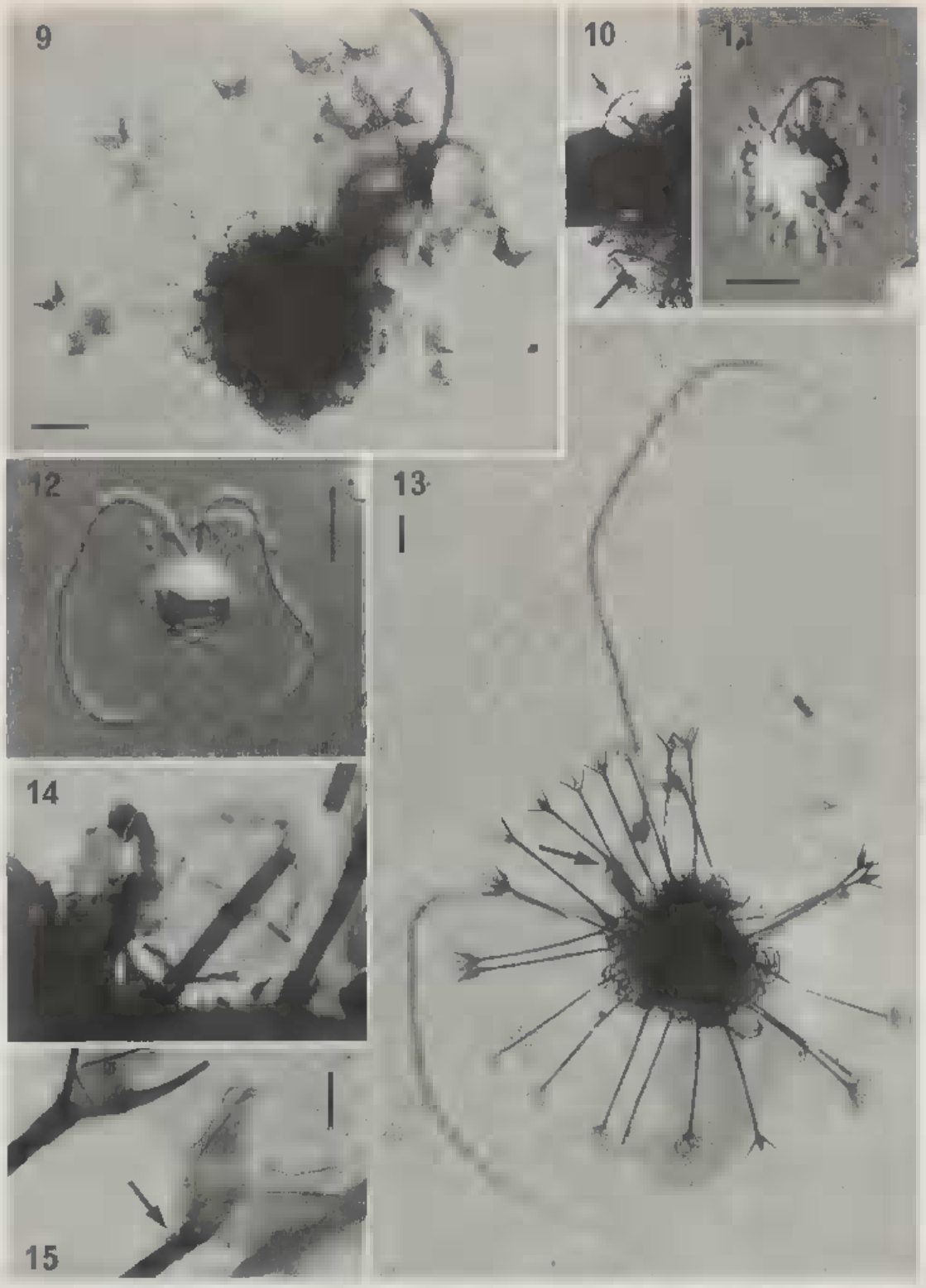
ØSTERGAARD J.B., 1993 — *Nanoplanktoniske coccolithophorer (Prymnesiophyceae) og nanoplanktoniske, loricabærende choanoflagellater (Acanthoecidae) i farvandet omkring Godhavn, Grønland*. M.Sci. thesis, University of Copenhagen, 157 p.



Figs 2-4. *Papposphaera lepida* from California; shadowcast whole mounts for TEM. Fig. 2. Cell with flagella. Fig. 3. High magnification of coccosphere. Notice the conspicuous size difference between apical (arrow) and antapical (arrowhead) cell end pappoliths. Fig. 4. Detail of process appendage (x 20,000). Scale bars: 1 μm (Fig. 3); 2 μm (Fig. 2).

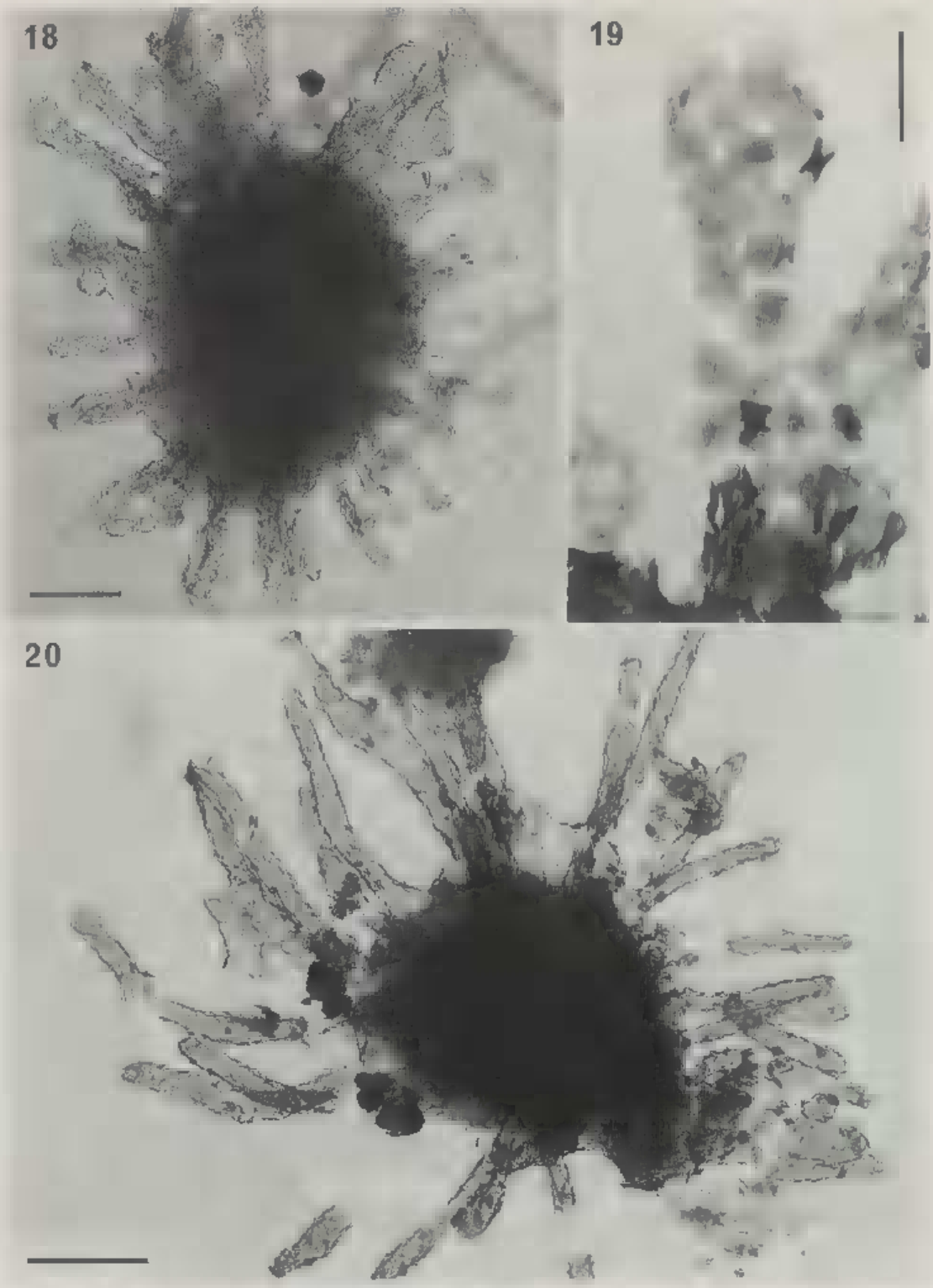


Figs 5-8. *Papposphaera lepida* from Mexico; shadowcast whole mounts for TEM. Fig. 5. Coccosphere with square process appendages only. Notice the coiling haptonema (arrow). Fig. 6. Details of process appendage. Notice wristlet and the displaced triangles forming a square central aperture (arrow). Fig. 7. Detail of pappolith base-plate calcification (arrow). Fig. 8. Coccosphere comprising a single discoid process appendage (arrowhead). Notice the two flagella and the conspicuous size difference between apical and antapical cell end pappoliths. Scale bars: 0.5 μm (Figs 6-7); 1 μm (Figs 5, 8).

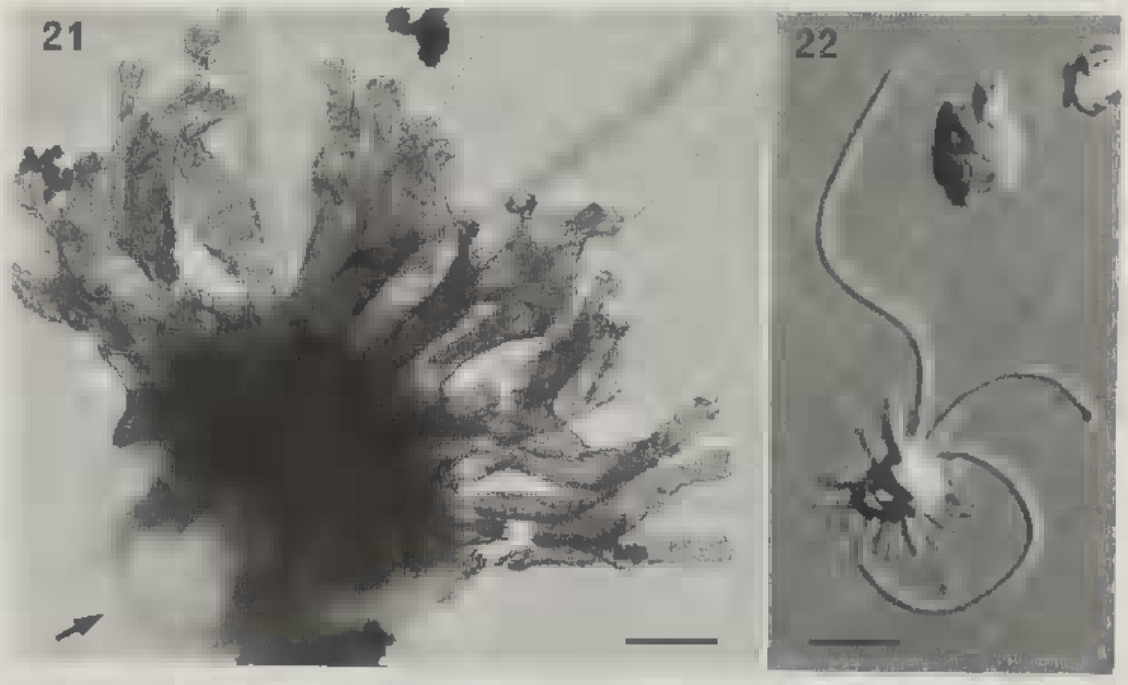


Figs 9-15. *Papposphaera bourrellii* sp. nov. from California (Figs 9-12), and San Juan Islands (Figs 13-15). Shadowcast whole mounts for TEM (Figs 9-10, 13-15); light micrographs (Figs 11-12). Fig. 9. Weakly calcified cell with intact flagellum and haptonema and conspicuous calyx-like process appendages. Fig. 10. Detail from normally calcified specimen; pappolith with cruciform base-plate calcification is indicated (arrow). For scale bar see Fig. 9. Figs 11-12. Light micrographs of Californian specimens (Fig. 11 is reproduced with permission from the Systematics Association). Fig. 13. Holotype (iconotype) specimen from McConnell Island with flagella and haptonema (arrow). Fig. 14. Detail of base-plate calcification (see text for further explanation). For scale bar see Fig. 15. Fig. 15. Detail of process appendages. The arrow points to the collar that is part of the interlocking devices between shaft and process appendage. Scale bars: 0.25 µm (Figs 14-15); 1 µm (Figs 9-10, 13); 5 µm (Figs 11-12).

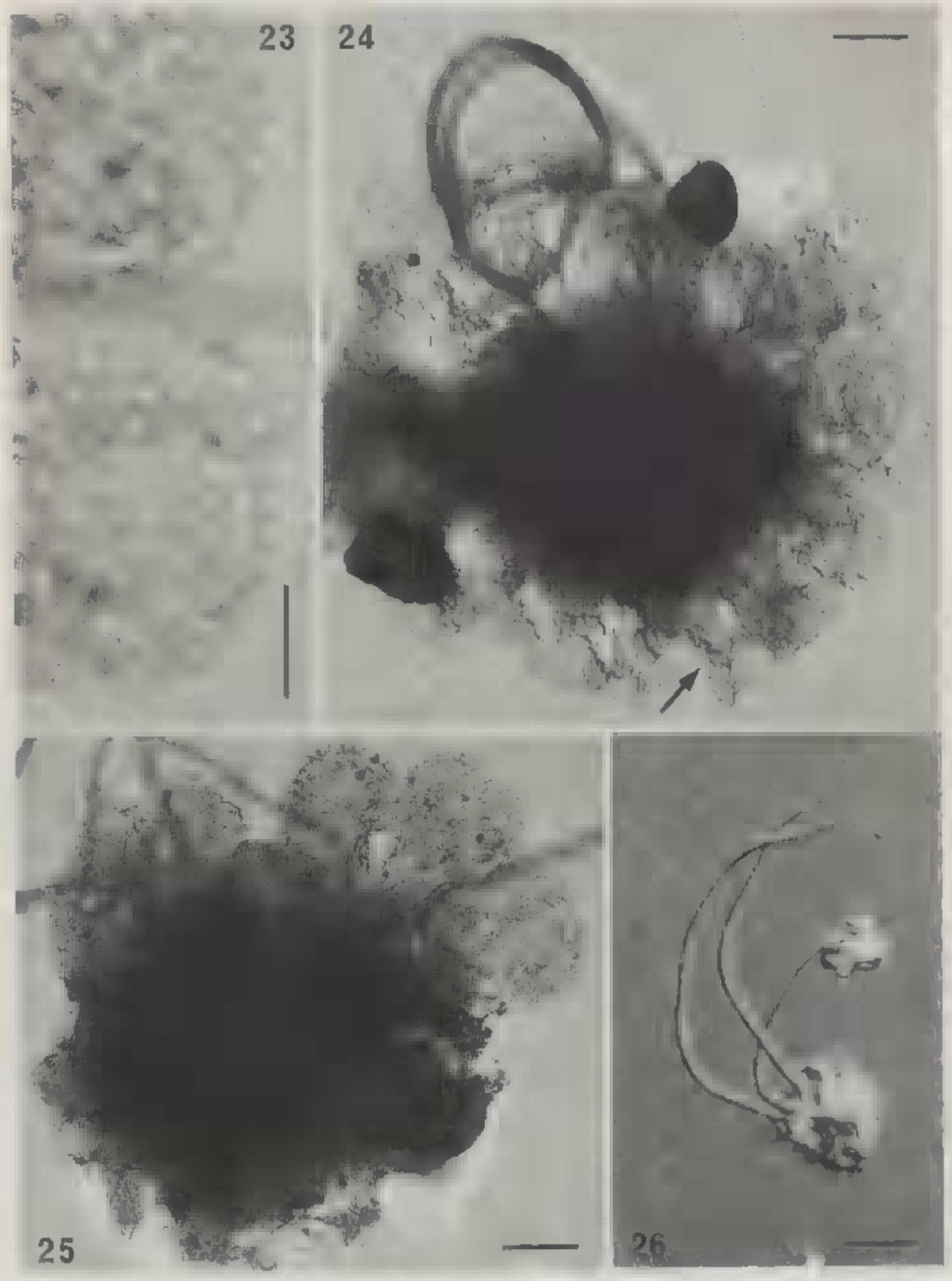
Fig. 16. *Papposphaera hourrellii* from Mexico. Light micrograph of specimen with flagella and haptonema; notice that the species identification is uncertain. Fig. 17. *Papposphaera* sp. 1 from Mexico. Shadowcast whole mount for TEM showing details of pappoliths. The arrow points to elements from the coccolith base plate rim. Scale bars: 0.25 µm (Fig. 17); 5 µm (Fig. 16).



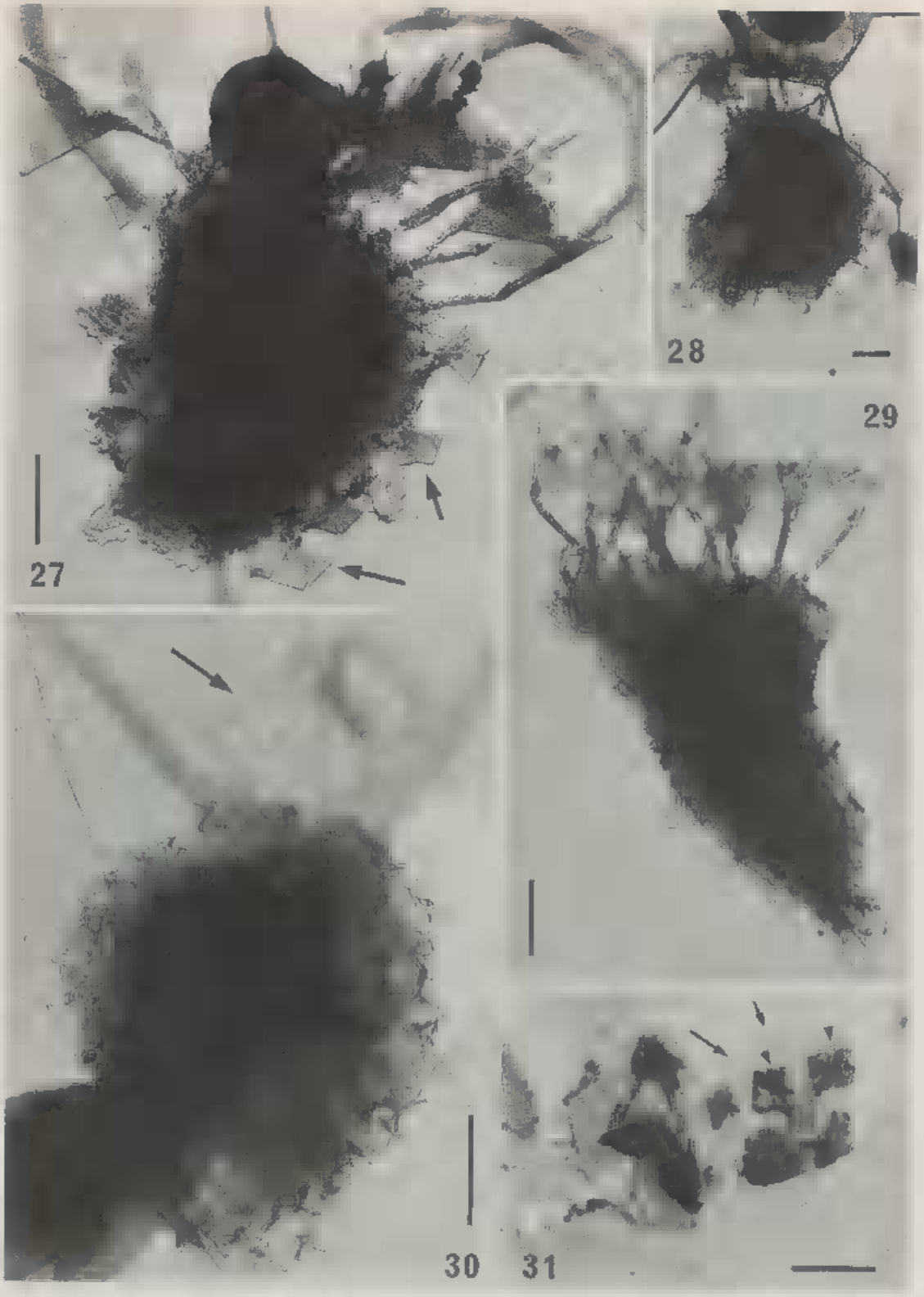
Figs 18-20. *Papposphaera* sp. 2 ("*Turrisphaera* phase") from Mexico; shadowcast whole mounts for TEM. Fig. 18. Cell with both flagella intact and ■ complete coverage of turriform coccoliths; notice the difference in overall coccolith length from one cell pole to the other. Fig. 19. Detail of coccolith showing hexagonal crystallites. Fig. 20. Cell with very long, thin and irregularly bending coccoliths. Scale bars: 0.25 µm (Fig. 19); 1 µm (Figs 18, 20).



Figs 21-22. *Papposphaera* sp. 2 ("*Turrisphaera* phase") from California; shadowcast whole mount for TEM (Fig. 21) and Nomarski light micrograph of dried cell (Fig. 22). Fig. 21. Broken coccosphere. The apical pole coccoliths are irregularly shaped and with weakly developed, distal, unilateral proliferations. An underlayer of minute, unmineralised scales is exposed at one end of the cell (arrow). Fig. 22. Complete cell with flagella and haptonema. Reproduced with permission from the Systematics Association. Scale bar: 1 μm (Fig. 21); 5 μm (Fig. 22).



Figs 23-26. *Papposphaera* sp. 3 ("*Turrisphaera* phase") from California; shadowcast whole mounts for TEM (Figs 23-25) and Nomarski light micrograph of dried cell (Fig. 26). Fig. 23. Detail of coccolith (from Fig. 25) showing the hexagonal crystallites. Fig. 24. Weakly calcified specimen. The arrow points to a symmetrically shaped coccolith from the antapical cell end. Fig. 25. Complete cell with flagella and haptonema intact; notice the unilateral, flaring proliferations of the apical cell end coccoliths. Fig. 26. Whole cell with flagella and haptonema. Scale bar: 0.5 µm (Fig. 23); 1 µm (Figs 24-25); 5 µm (Fig. 26).



Figs 27-29. *Pappomonas flabellifera* var. *flabellifera* from Mexico (Fig. 27) and California (Figs 28-29); shadowcast TEM whole mounts. Fig. 27. Complete cell; arrows point to antapical cell end pappoliths with reduced central processes. Fig. 28. Cell with numerous, reduced, antapical appendages. Fig. 29. Complete cell. Reproduced with permission from the Systematics Association. Scale bar: 1 μm (Figs 27-29).

Figs 30-31. *Polycrater galapagensis* from Mexico (Fig. 31) and California (Fig. 30); shadowcast whole mounts for TEM. Fig. 30. Cell complete with flagella and haptonema. Reproduced with permission from the Systematics Association. Fig. 31. Details of single coccoliths. Arrows point to petal-like elements forming a bowl. The subtending cruciform structure is identified with arrowheads. Scale bar: 0.25 μm (Fig. 31); 1 μm (Fig. 30).